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Salmonella enterica Serotype enteritidis in Table Egg Layer House Environments and in Mice in U.S. Layer Houses and Associated Risk Factors

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SUMMARY. Prevalence was estimated for *Salmonella enterica* serotype *enteritidis* (SE) in layer house environments (n = 200 layer houses) and house mice (n = 129 layer houses) in 15 states throughout the United States. Environmental swabs were collected from manure, egg belts, elevators, and walkways. Live-catch rodent traps were placed for 4–7 days. Swabs and house mice were submitted to the laboratory for bacterial culture. Overall, 7.1% of layer houses and 3.7% of mice were culture positive for SE. The highest prevalence was in the Great Lakes region of the United States, and no SE was recovered from houses or mice in the southeast region. Presence of SE in layer houses was associated with age/molting, floor reared pullets, and number of rodents trapped. Cleaning and disinfecting houses between flocks was associated with a reduced risk. The prevalence of SE in mice from environmentally positive houses was nearly four times that of mice from environmentally negative houses.

RESUMEN. Factores de riesgo asociados con la Salmonella enterica serotipo enteritidis en el medio ambiente de casetas de ponedoras comerciales y en ratones presentes en las casetas de ponedoras comerciales.

En 15 estados de Estados Unidos de América, se estimó la prevalencia de *Salmonella enterica* serotipo *enteritidis* en el medio ambiente de 200 casetas de ponedoras comerciales y en los ratones habitantes de 129 casetas. Se tomaron hisopos de la materia fecal, poleas móviles y pasillos. Se colocaron trampas para ratones durante 4 a 7 días. Tanto los hisopos como los ratones fueron enviados al laboratorio para cultivo bacteriológico. El 7.1% de las casetas de ponedoras y el 3.7% de los ratones fueron positivos a *S. enterica* serotipo *enteritidis.* La mayor prevalencia ocurrió en la región de los grandes lagos. No se aisló la Salmonella a partir de los galpones o de los ratones en la región sureste de los Estados Unidos. La presencia de la *S. enterica* serotipo *enteritidis* en las casetas estuvo asociada con la edad, la muda forzada, el levante en el piso y el número de roedores atrapados. La limpieza y desinfección de las casetas estuvo asociada con un riesgo reducido. La prevalencia de *S. enterica* serotipo *enteritidis* en los ratones obtenidos en las casetas con medio ambiente positivo fue casi cuatro veces mayor comparado con las casetas con medio ambiente negativo.

Key words: layers, mice, *Salmonella enteritidis,* prevalence

Abbreviations: ARS = Agricultural Research Service; NAHMS = National Animal Health Monitoring System; NASS = National Agricultural Statistics Service; NVSL = National Veterinary Services Laboratories; SE = *Salmonella enterica* serotype *enteritidis*; VMO = Veterinary Medical Officer; VS = Veterinary Services

Salmonella species can cause diarrheal illness in humans and animals and is a safety issue for foods from all animal sources. There are over 2000 *Salmonella* serotypes (12). *Salmonella typhimurium* and *Salmonella enterica* serotype *enteritidis* (SE) are the most common serotypes in the United States (20).

SE is the primary serotype of food safety concern from poultry sources. It is of particular concern in the United States to the egg layer industry because SE can infect the reproductive tracts of laying hens and subsequently infect eggs if the SE is deposited in or on the egg. Even though layers may harbor SE, they show no sign of infection, and the eggs they produce appear normal. The rate of egg contamination with SE is sporadic and is estimated between 1 and 11 contaminated eggs per 100,000 eggs laid (18).

The National Animal Health Monitoring System (NAHMS), a program of the United States Department of Agriculture, Animal and Plant Health Inspection Service, Veterinary Services (VS), was approached by the layer industry with a request for a national table egg layer study addressing the issue of SE. The Layers '99 study was the first NAHMS national study of the layer industry and was a cooperative effort between state and federal agricultural statisticians, animal health officials, university researchers, extension personnel, and table egg layer operators. The objectives of the study reported here were 1) to estimate the prevalence of SE in layer houses, 2) to describe possible risk factors associated with presence of SE, and 3) to estimate the prevalence of SE in mice found in layer houses.

MATERIALS AND METHODS

Sampling. The design and implementation of the Layers '99 study was similar to previous NAHMS studies (9,10,15). The goal for NAHMS national studies is to include states that account for at least 70% of the animal and farm population in the United States. The National Agricultural Statistics Service (NASS) Layers and Egg Production, 1997 Summary (19) was used to determine state ranking for table egg layers. All states with 4.0% or more of the U.S. table egg layers were included in the study. In addition, five states with less than 4% of layers were added to provide better geographic coverage (Missouri, Washington, North Carolina, Arkansas, Alabama), resulting in a total of 15 states participating, representing 82% of 1997 U.S. table egg layers.

The NASS maintains a list of all egg-laying operations with 30,000 or more laying hens that is the basis for estimating monthly egg production. An operation may have one farm or multiple farms. The individual farms may have fewer than 30,000 layers, but the total layers for all farms associated with a company must equal or exceed 30,000 to be included on the list. All operations that had 30,000 or more laying hens (20 wk of age or older) in the 15 selected states were eligible to participate in the study. A total of 328 operations were eligible to participate.

NASS enumerators made the first personal contact with the operations. Enumerators visited company headquarters except for single-farm operations, where the farm was visited. If a company had farms in more than one state, each state was treated as a separate operation (assigned a unique operation identification code), and the NASS enumerator contacted the person who routinely reported inventory data for the company in that state to NASS. For multiple-farm operations, the NASS enumerator selected a random sample of farms to participate with a random numbers table. All farms were selected for operations with 10 or fewer farms. If the operation had 11–29 farms, 10 farms were selected. If there were 30 or more farms, 15 farms were selected.

Data collection. The NASS enumerator administered a layers management report. This questionnaire (available upon request) was limited to items that could more readily be answered by company headquarters than by personnel on a farm (e.g., pullet sources, feed sources). A separate questionnaire was completed for each farm. If an operation was willing to continue to the next stage of the study, a consent form was signed.

Farms for which the operation had signed a consent form were contacted by VS for the second phase (on farm) of the study. Federal and state veterinary medical officers (VMOs) or animal health technicians contacted each farm from participating operations, explained the program, and administered a questionnaire that could most readily be answered by farm personnel (e.g., housing, biosecurity).

The farms were visited one additional time for collection of biologic samples. Environmental culturing was offered to all farms. Typically, one house per farm was randomly selected for culturing; however, on a few large farms, more than one house was selected. Samples were collected from surfaces distributed evenly throughout the house including manure (five samples per house), egg belts (five samples per house), elevators (five samples per house), and walkways (two samples per house). If the house did not have egg belts or elevators, then 10 samples were collected from cage floors. Each sample consisted of two 4- × 4-inch gauze swabs. Swabs were moistened with skim

milk prior to sample collection. Samples were placed in whirl-pak bags containing skim milk and shipped overnight on ice to the Agriculture Research Service (ARS) in Athens, GA, for culture. Information about the flocks and houses being sampled was recorded on a clinical evaluation record.

Rodent collection was offered to 150 farms that also participated in environmental sampling. Live-catch rodent traps (Victor Tin Can; Woodstream, Lititz, PA) were placed in each house. VMOs were provided guidelines as to trap placement and identification of locations in the layer houses with potential high rodent activity. VMOs returned 4–7 days later to count the number of rodents caught, and live rodents were euthanatized with dry ice. House mice (*Mus musculus*) were placed in large whirl-pak bags and shipped overnight on ice to National Veterinary Services Laboratories (NVSL), Ames, IA, for culture of internal organs. Other species (e.g., deer mice) were not tested because of human safety concerns due to the association with hantavirus (3). The number of rodents trapped, number of house mice submitted, trap location, number of days the trap was set, and whether the trap had functioned properly were recorded on a rodent submission form.

Laboratory methods. Bacterial culture of manure has been described previously (10). Upon arrival at the ARS laboratory, all environmental samples were pre-enriched in buffered peptone water (Difco Laboratories, Inc., Detroit, MI) at 37 C overnight. Aliquots were transferred to gram-negative broth (Difco) and tetrathionate broth (Difco) that were incubated for 24 and 48 hr, respectively, at 37 C. Aliquots from these two selective enrichments were transferred to Rapport R10 medium (Difco), incubated at 37 C overnight, then streaked onto xylose-lysine-tergitol 4 agar (Difco) and brilliant green sulfa agar (Difco) plates and incubated 24 hr at 37 C. Presumptive positive colonies were inoculated into triple sugar iron agar (Difco) and lysine iron agar (Difco) for biochemical confirmation. Up to three colonies per plate were selected, and isolates were serogrouped by agglutination with *Salmonella* O antiserum (Difco). Group D isolates were sent to NVSL for serotyping.

Mice were processed by NVSL under a biological safety cabinet. They were taken from whirl-paks and placed in a quaternary ammonium disinfectant (Roccal II) to disinfect surface fur. From the disinfectant, mice were opened along the ventral midline with sterilized instruments. With another set of sterilized instruments, the intestines were aseptically removed. Intestines from up to five mice from the same farm were pooled for culture. Intestines were macerated and placed into tetrathionate enrichment broth. Enrichments were incubated at 42 C and plated onto brilliant green agar with novobiocin and xylose-lysine-tergitol 4 agar at 24 and 48 hr. All plates were incubated at 37 C and read at 24 and 48 hr. Up to five typical *Salmonella* colonies were picked from plates to triple sugar iron and lysine iron agar slants that were incubated at 37 C for 24 hr. Enrichments were held an additional 5 days at 25 C. For mice from which no *Salmonella* were isolated from the 24- or 48-hr enrichment, 100 μl of tetrathionate was transferred to 10 ml of Rappaport-Vassiliadis broth and incubated at 37 C for 24 hr. Aliquots of this broth were plated as described above and read at 24 and 48 hr. Colonies with reactions typical of *Salmonella* were screened with grouping sera, and group D cultures were serotyped.

Data analysis. Data were entered into a SAS data set (SAS Institute, Inc., Cary, NC). Data validation checks were performed, and VS field staff followed up with producers where necessary. Data were weighted in order to make inferences to the population (2) (operations with 30,000 or more laying hens in the 15 states). Because all operations that had 30,000 or more laying hens in the 15 selected states were eligible to participate in the study, the probability of selection (selection weight) was one for all operations. This selection weight was adjusted for nonresponse as the sum of weights for all eligible operations divided by the sum of weights for responding operations within state and size group strata. For each participating farm, a farm-level weight was created, equal to the operation weight multiplied by an expansion factor as follows: farm weight = operation weight × (number of farms in the operation/number of operation's farms participating). This weight was adjusted in a similar manner for nonresponse at the VS phase. For the environmental sampling results, the farm level weight was expanded to account for the number of houses the farm had *vs.* the number of houses sampled.

Summarization and estimation for questionnaire data and environmental sampling results were performed with SUDAAN software, which was specifically designed to analyze data from multistage complex survey designs. Odds ratios and *P*-values were obtained by modeling each variable by logistic regression. Region and flock size were included as covariates to simultaneously adjust for the potential effects of these variables while evaluating the other variables of interest.

Because of laboratory limitations, rodent trapping was not offered to all farms. The number of houses allowed to participate in each region was roughly proportional to the size of the layer industry in that region, and, therefore, the mouse prevalence estimates were not weighted. Standardized rodent index was calculated as follows: rodent index = total number of rodents trapped × (7/number of days) × (12/number of functional traps), so that all houses were standardized to the equivalent of having 12 traps function for 7 days. SE prevalence in mice was esti-

mated by previously described methods for prevalence estimation from pooled samples (13). Rodent culture results were summarized with SAS software. Likelihood analysis modeling of rodent culture results by region was performed with @Risk software.

RESULTS

Response rates. The sample for Phase I included 328 operations that were considered eligible to participate. Of the 328 eligible operations, 208 operations agreed to participate (63%). The primary reasons for declining to participate included not time (n = 58), not wanting outside people on the operation (n = 31), and not wanting to be involved with a government veterinarian (n = 25). These 208 operations provided information on 526 individual farms. Consent was given to contact 393 of these farms for the second phase of the study (75%). Of the 393 farms contacted by VS, 11 were ineligible (no longer in business). Of the 382 eligible farms, 252 participated in the VS phase of the study (66%). The participating farms by region included west (102 farms), central (58 farms), southeast (65 farms), and Great Lakes (27 farms).

Only 27 of the 142 participants in the Great Lakes region continued on to the second phase of the study. In order to get some measure of the response bias caused by the poor participation of the Great Lakes region in this phase of the study, the small sample from this region for the VS phase of the study was compared with the relatively large sample this region provided for the NASS phase. The 27 VS-phase participants were similar to the larger NASS sample from the Great Lakes region in terms of size (35.0% of participants had 100,000 or more layers in each phase), testing feed for SE (43.8% of Phase I participants, and 51.0% of Phase II participants tested feed), and vaccination practices (percentages of participants that vaccinated against laryngotracheitis, *Mycoplasma gallisepticum,* fowl pox, *S. enteritidis,* and avian infectious coryza were 76.3% *vs.* 74.6%, 16.1% *vs.* 23.7%, 91.5% *vs.* 87.1%, 10.2 *vs.* 7.7%, and 10.4% *vs.* 0 for Phase I and Phase II, respectively).

A total of 200 houses provided environmental samples for culture, and rodents were collected from 129 of these houses.

Environmental culture results. Overall, SE was isolated from 7.1% of layer houses. Regional prevalence estimates were 0 in the southeast, 9.0% (standard error = 7.2) in the central region, 4.4% (standard error = 2.5) in the west, and 17.2% (standard error = 13.7) in the Great Lakes region. The standard error for the Great Lakes region estimate is large because of a small sample size as a result of low participation in this region.

Approximately 4% of houses with fewer than 100,000 layers were environmentally positive for SE, whereas 16.5% of houses with 100,000 or more layers were environmentally positive for SE. Environmentally positive houses had a mean of 109,777 layers (median = 120,000) *vs.* a mean of 64,346 layers (median = 54,000) in environmentally negative houses.

Nearly one-half of the positive flocks were identified via the egg belt (47.8%) or elevator (45.2%) samples. Fewer flocks were identified via manure swabs (16.9%) and walkways (18.1%). Sixty percent of positive houses had only one positive sample, and no house had more than two positive samples.

The average rodent index in SE-positive houses (38.9) was more than twice that of negative houses (16.7). A total of 2.0% of flocks with a rodent index less than 20 were positive for SE, compared with 10.1% of flocks with a rodent index of 20 or more. After adjusting for region and flock size, houses with a standardized rodent index of 20 or more were nearly nine times more likely to have SE found within the house than were houses with a rodent index of less than 20 (odds ratio = 8.9, P = 0.04).

Potential factors related to presence of SE are shown in Tables 1–3. The odds of a flock having at least one environmental sample testing positive for SE was evaluated for several flock characteristics (Table 1), farm management practices (Table 2), and cleaning and disinfecting practices (Table 3). These variables were modeled with region and flock size as covariates to adjust for possible confounding influences. Other potential confounders may exist, but because of the low number of positive flocks, additional covariates could not be modeled.

Flocks that were 0–16 wk postmolting were 9.3 times more likely to test positive compared with flocks that were 60 or more weeks of age and unmolted, but flocks more than 16 wk postmolt had very little increased risk. Younger

Table 1. Percentage of flocks positive for SE (on the basis of environmental cultures) by flock characteristics.

Flock characteristic	% Positive	Standard error	Odds ratio	*P*-value
Breed/strain				0.03
Hy-line	5.2	3.7	0.21	
Other white	12.3	5.7	1	
Brown	0.0		Too few	
Age/molt				0.02
Less than 60 wk of age, not molted	8.0	4.5	4.7	
60 wk or more of age, less than 16 wk postmolt	11.3	6.2	9.3	
60 wk or more of age 16 wk or more postmolt	3.9	3.6	1.4	
60 wk or more of age, not molted	4.9	4.3	1	
Any concurrent disease				0.12
Yes	11.1	6.3	3.4	
No	5.1	2.5	1	
Flock health				0.16
Excellent	4.5	2.6	0.3	
Good/fair	10.1	5.6	1	
SE vaccination (this flock)				
Yes	0.0		Too few	
Don't know	2.8	2.5		
No	8.7	4.6		
Competitive exclusion product administered (this flock)				
Yes	0.0		Too few	
Don't know	2.1	1.9		
No	8.5	4.5		

flocks (less than 60 wk of age) were 4.7 times more likely to test positive than older, unmolted flocks. Flocks that were reported to be in excellent health and that had no concurrent diseases were less likely (although marginally insignificant) to test positive than other flocks.

Flocks that had been primarily floor reared as pullets were 5.9 times more likely to test positive for SE than were flocks that had been cage reared. The SE prevalence was slightly higher for flocks on farms that fed poultry by products; however, this difference was not statistically significant. None of the flocks tested positive on farms that fed feeds without animal products. Flocks where pests such as flies, wild birds, and rodents had access to the feed prior to it being fed (e.g., hoppers, lines) were 6.2 times more likely to test positive. Flocks where visitors were allowed in the layer houses had a fivefold increased odds of testing positive. None of the houses that used a flush system to handle manure tested positive compared with 13.4% of houses with high rise or deep pits for manure. The association with manure handling method may be related to the regional distribution of these practices (16). For houses with pits, the SE prevalence was lower (although not statistically significant) for those that had cleaned out the pit within the previous 6 mo (3.4%) compared with those that had gone a longer time since cleaning the pit (15.7%). Additional factors evaluated and not found to be significant included testing the feed for SE, the age of the house, and the square inches of space per bird.

None of the houses tested positive for SE on farms where the feeders or hoppers were cleaned and disinfected between flocks. Also, no house tested positive where cages, walls, and ceilings were washed between flocks, whether or not they were fumigated. Houses that were fumigated between flocks had a lower prevalence of SE than houses that were neither fumigated nor washed. A reduced risk was not identified in this study for dry cleaning cages and walls or for cleaning egg belts and elevators.

Flocks of the Hy-line breed had a lower prevalence of SE (5.2%) than other white egg breeds combined (12.3%). There were too few flocks of any other specific white egg breed to

Table 2. Percentage of flocks positive for SE (on the basis of environmental cultures) by farm management factors.

Farm management factor	% Positive	Standard error	Odds ratio	*P*-value
Floor reared as pullets				0.04
Yes	10.5	8.3	5.9	
No	5.4	2.7	1	
Feed contains poultry by-products				0.67
Yes	8.6	7.2	1.5	
No	6.0	3.1	1	
Feed contains animal products				—[A]
Yes	8.9	4.4	1	
No	0.0		<1	
Water chlorinated				—
Yes	0.0		<1	
No	8.3	4.6	1	
Pests have access to feed (prior to feed trough)				0.03
Yes	9.6	4.6	6.2	
No	5.8	4.9	1	
Visitors allowed (nonbusiness)				0.04
Yes	17.0	10.3	5.0	
No	3.6	2.2	1	
Manure handling method				—
High rise/deep pit	13.4	7.6	2.3	
Flush system	0.0		<1	
Other (shallow pit, manure belt, and scraper)	4.1	2.3	1	
Pit cleaned out in previous 6 mo				0.20
Yes	3.4	3.3	0.26	
No	15.7	9.3	1	

[A] *P*-value was not generated where no positive flocks were identified for one level of the variable.

evaluate separately. None of the brown egg flocks tested positive, but these flocks were too few to evaluate statistically ($n = 15$). None of the flocks that had been vaccinated against SE ($n = 17$), that had been given a competitive exclusion product ($n = 6$), or that drank chlorinated water tested positive ($n = 27$); however, very few flocks received these practices and, therefore, these factors could not be evaluated statistically.

Table 3. Percentage of flocks positive for SE (on the basis of environmental cultures) by cleaning and disinfecting practices.

Cleaning and disinfecting between flocks	% Positive	Standard error	Odds ratio	*P*-value
Feeders				—[A]
Yes	0.0		<1	
No	11.2	5.3	1	
Hoppers				—
Yes	0.0		<1	
No	10.1	4.9	1	
Cages, walls, ceiling				—
Wash and fumigate	0.0		<1	
Wash only	0.0		<1	
Fumigate only	5.3	3.2	1	
Neither	12.2	6.5	3.2	

[A] *P*-value was not generated where no positive flocks were identified for one level of the variable.

Table 4. Percentage of house mice culture positive for SE by region, flock size, and environmental culture status.

Factor	% mice positive
All mice	3.7
Region	
Great Lakes	7.3
Southeast	0.0
Central	2.3
West	3.7
Flock size (number of layers)	
<100,000	3.5
≥100,000	3.9
Environmental culture status	
Positive	11.2
Negative	2.9

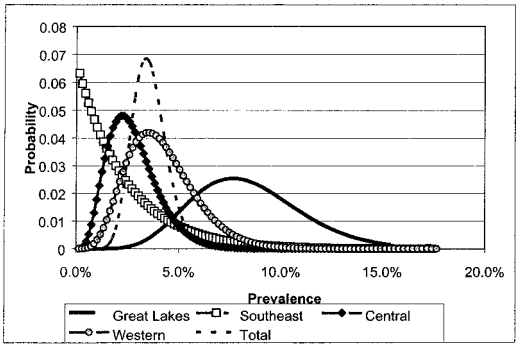

Fig. 1. Probability distribution of SE prevalence in house mice by region.

Mouse culture results. Overall, 3.7% of house mice cultured were positive for SE (Table 4). None of the mice collected from the southeast region were positive. The Great Lakes region had the highest SE prevalence in mice (7.3%). This regional distribution in mice was roughly consistent with the environmental results.

Because the rodent data analysis was unweighted, a standard error was not calculated. In order to put some bounds on the estimates, likelihood analysis was performed. This analysis gives a probability distribution for the estimates, i.e., Fig. 1 shows the probability of obtaining the results in our data if the true prevalence were at various levels. The range around the mouse prevalence estimate in the west region (3.7%) was fairly narrow, whereas the estimate for the Great Lakes region (7.3%) had a much wider possible range. The probability of obtaining our results (no positive mice) in the southeast region peaked at a true prevalence of 0, and the probability of obtaining this result decreased with a true prevalence greater than 0.

Although there were some environmentally positive houses with no positive mice and environmentally negative houses with positive mice, the prevalence of SE in house mice from environmentally positive houses was nearly four times that of mice from environmentally negative houses.

DISCUSSION

The overall SE prevalence found in this study was lower than that in a previously reported spent hen survey (4). Our sampling design did not allow identification of flocks with very low levels of contamination. Therefore, risk factors identified here are related to a high level of contamination (contamination sufficient to be detected at this level of sampling). Flocks with high levels of manure contamination are more likely to produce contaminated eggs and, thus, pose the greatest risk to human health (7).

Participation in this study was entirely voluntary on the part of producers, and, therefore, confidentiality of data was crucial to the success of the study. Despite assurances of confidentiality, producers in the Great Lakes region may have been wary of providing samples to be tested for SE, resulting in an imprecise prevalence estimate (large standard errors) due to poor participation in that region. The impact of participation rate is also apparent in the mouse prevalence estimates. Because standard errors could not be calculated, bounds around the prevalence estimates were evaluated graphically via likelihood analysis modeling. The Great Lakes region has a very wide distribution of possible prevalences, given our data, whereas the other regions, which had higher response rates, have a much narrower prevalence range.

To assess the bias caused by the low response rate, data collected in the Great Lakes region during Phase I of the study was compared for the large Phase I sample *vs.* the small sample that continued on to Phase II. Response rate was not related to these variables (farm size, testing feed for SE, and vaccination practices). Our prevalence estimate for the Great Lakes region (17%) was similar to that found in a 1995

Pennsylvania pilot study via manure swabs (13% of houses) (22).

The reason SE was absent in the southeast region is not clear. Studies in other livestock commodities (equine [15], dairy [9], feedlot [10]) have shown the southeast region to have a higher prevalence of *Salmonella* species in general. Yet the serotype SE seems to be absent, despite the presence of risk factors in the southeast region such as molting (17) and floor rearing (16). Possible explanations include a lack of introduction of this particular serotype in the southeast region or an inability of this particular serotype to tolerate a warmer climate. Other studies of laying hens in the southeast region also found very low prevalences (1,21).

Because farms participating in rodent trapping were part of a convenience sample subset of the larger Phase I sample, analysis was not weighted. To optimize regional representation, the number of houses targeted to participate in each region was roughly proportional to the size of the layer industry in that region. Not only did environmentally positive houses have higher numbers of rodents present, the prevalence of SE in house mice from environmentally positive houses was nearly four times that of mice from environmentally negative houses. Other studies have also found a higher prevalence of SE in mice from contaminated premises compared with mice from "clean" premises (5,6). These results support the theory that mice may both amplify and spread SE in layer houses. Henzler and Opitz (8) have shown that up to 10^5 colony-forming units of *Salmonella* may be present in a single mouse fecal pellet (8).

Molted flocks were more likely to test positive for SE compared with similar aged unmolted flocks, which is consistent with results of a risk assessment conducted by Food Safety and Inspection Service (18). Because of the cross-sectional design of this study, molting was dichotomized at $\geq$16 wk postmolt and $<$16 wk postmolt. A decreased risk was found in the $\geq$16 wk postmolt group compared with flocks within 16 wk of molt. A longitudinal study with repeated sampling would be needed to identify at what point postmolt SE shedding peaks and then decreases.

Vaccination (6) and competitive exclusion products (especially when combined with antibiotic treatment) (14) show promise in research settings. Vaccinated flocks that participated in the Pennsylvania Quality Assurance program had a lower SE prevalence than did unvaccinated flocks (11). None of the flocks in this study that had been treated with a competitive exclusion product and none that had been vaccinated against SE tested positive; however, these practices were used in too few flocks to evaluate them statistically.

This study identified several management practices that are associated with the prevalence of SE, including molting, rodent control, restricting visitors in the layer houses, and cleaning and disinfecting between flocks. Competitive exclusion and vaccination may also have potential in SE control programs. Because large layer houses and houses in the Great Lakes region have the highest prevalence of SE, these operations should be particularly mindful of management strategies to reduce SE.

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